# Microbial source tracking

## An overview and potential advances with microarray technology

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# Why source tracking?

- Management and mitigation
- Better risk assessment

# Possible markers

- Bacteria – *E. coli*, *Enterococcus*, *Bacteroides*, *Bifidobacterium*
- Protozoa – *Cryptosporidium* spp. oocysts
- Virus – coliphages
- Chemical (caffeine, fecal steroids)
- Immunological targets (sIgA)

# Microbial Source Tracking (MST)

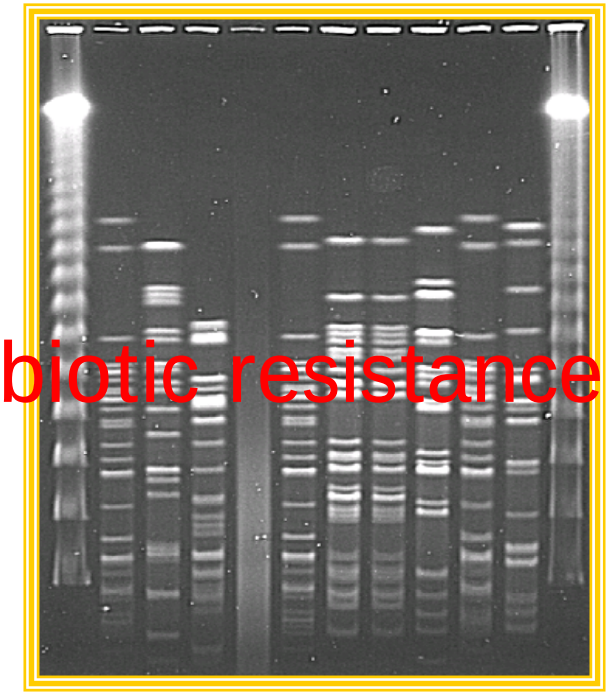
- Assumptions:
  - Host specificity exists
  - Ratios remain constant
  - No significant environmental replication
  - No significant environmental reservoirs

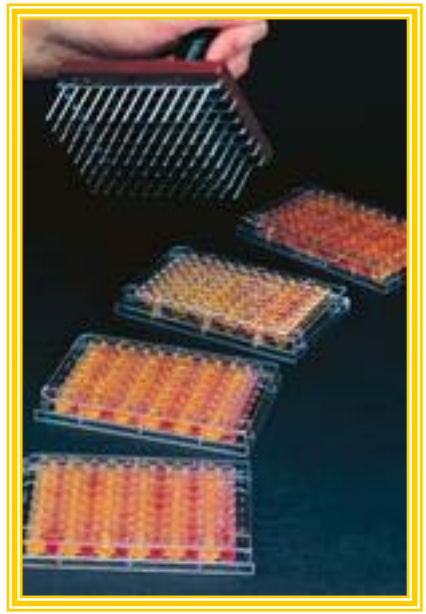
# Strategies with bacteria

- Library dependent
  - Antimicrobial resistance analysis
  - REP-PCR, PFGE, Ribotyping, RAPDs, DGGE, AFLPs
- Library independent
  - Host-specific genetic markers
- Microarrays

Phenotype, e.g., antibiotic resistance

Genotype, e.g., presence/absence of specific genes or "fingerprints"





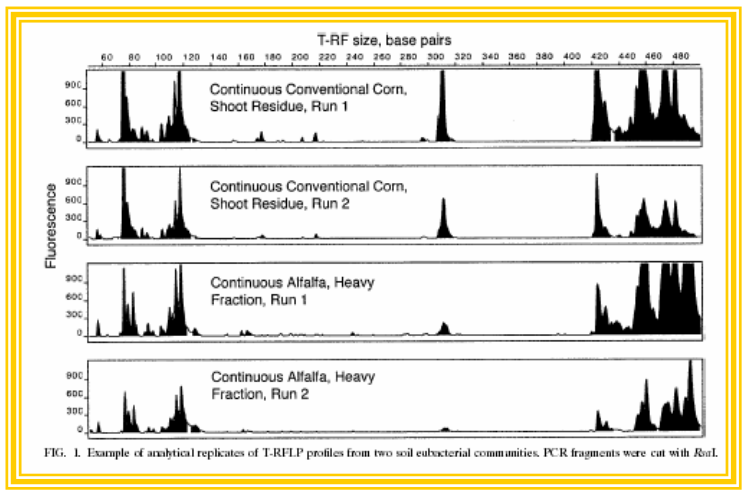
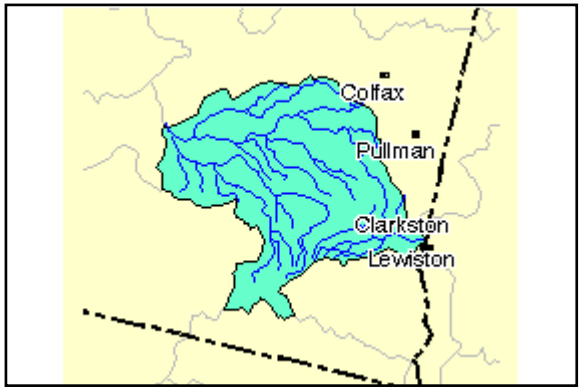


FIG. 1. Example of analytical replicates of T-RFLP profiles from two soil eubacterial communities. PCR fragments were cut with *Rsa*I.




People

Waterfowl

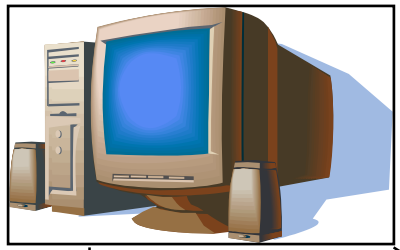
Cows

Dogs

Deer

Collect reference strains

Construct “library”



Generate classification function

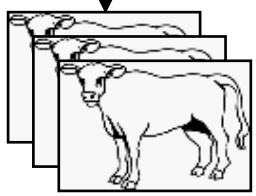
Identify source specific markers

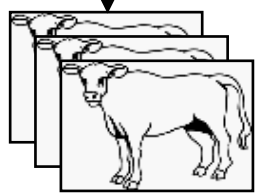
Collect unknown

Collect unknown

?

Y/N

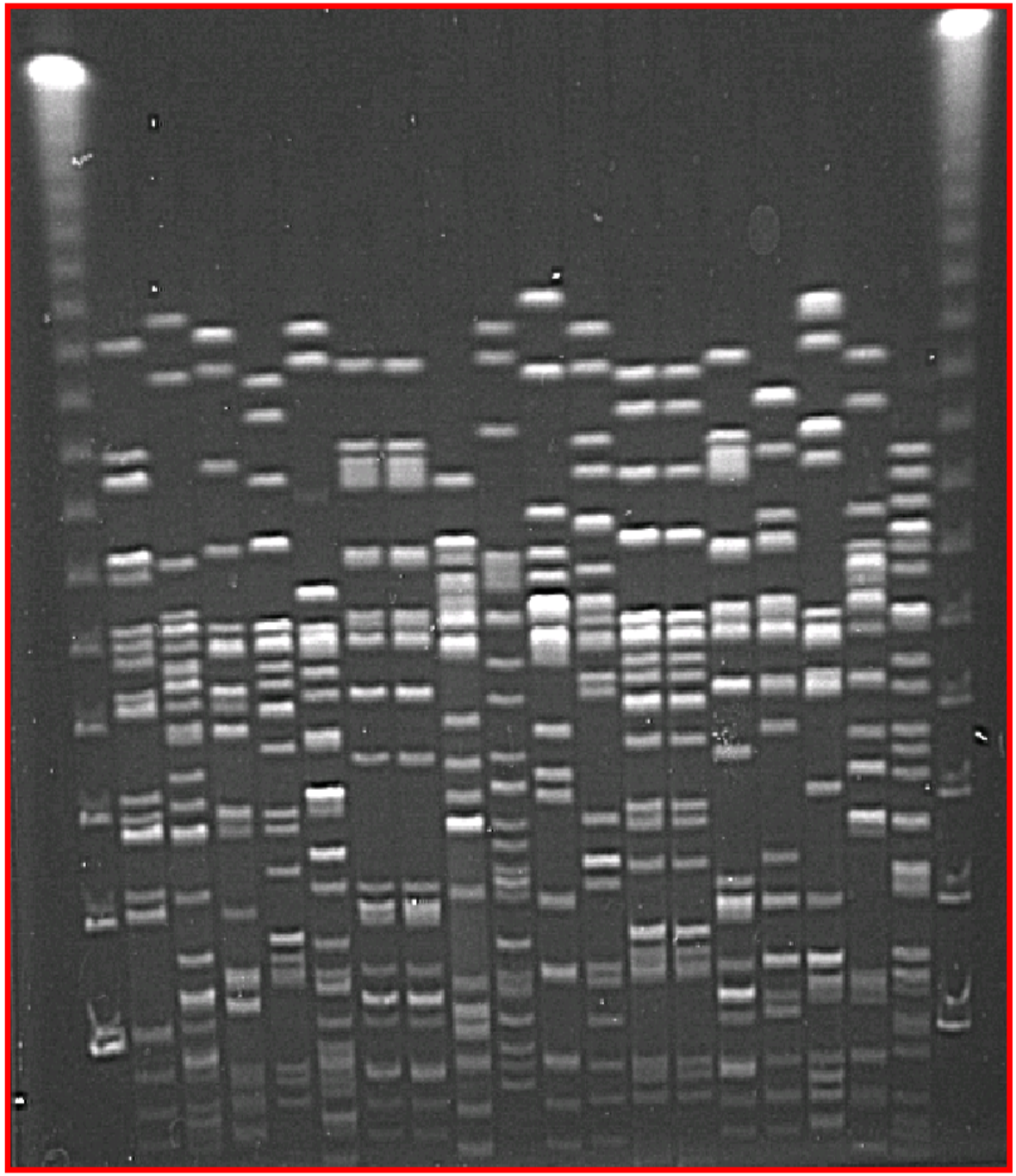




Wiggins et al. 2003. Use of antibiotic resistance analysis for representativeness testing of multiwatershed libraries. (Applied and Environmental Microbiology, 2003)

- Six watersheds (Virginia)
- 6,587 Enterococci
- Human, domesticated and wild animal sources
- Characterized using 10 drugs (3 levels)

| Source (n=6,587) | Human | Domestic | Wild |
|---|---|---|---|
| Human (n=1,970) | 63% | 19% | 18% |
| Domestic (n=3,345) | 20% | 54% | 26% |
| Wild (1,272) | 21% | 24% | 55% |



# Challenges

- Technical expertise
- Practical application
  - Sampling regime
  - Assay cost
  - Sensitivity, specificity
  - Assay format
  - Validation

# Strategies with bacteria

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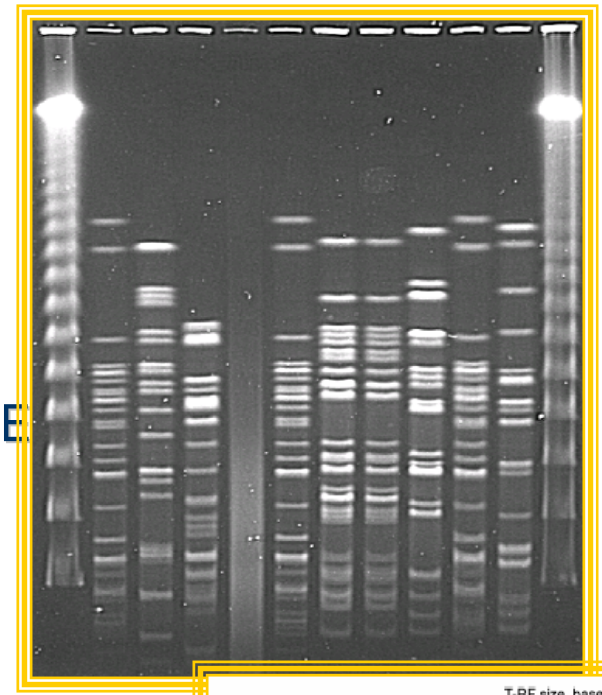
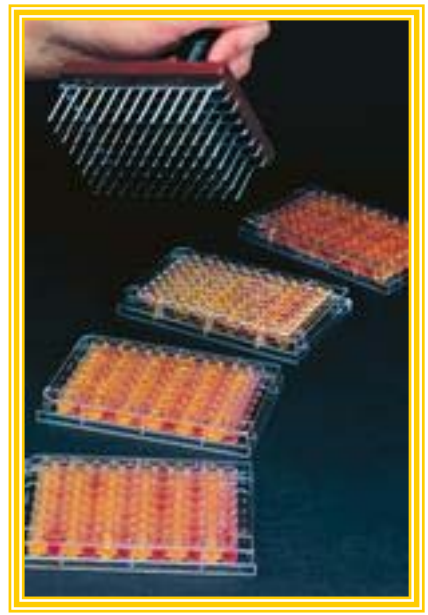
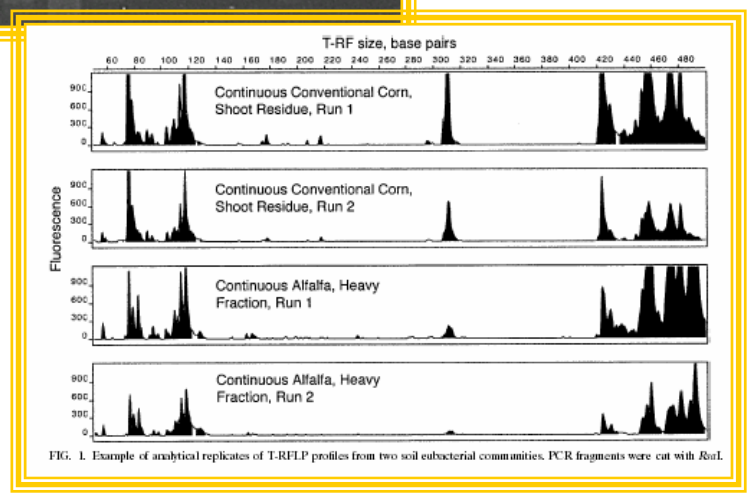
  - REP-PCR, PFGE, Ribotyping, RAPDs, DGGE, AFLPs
- Library independent
  - Host-specific genetic markers
- Microarrays







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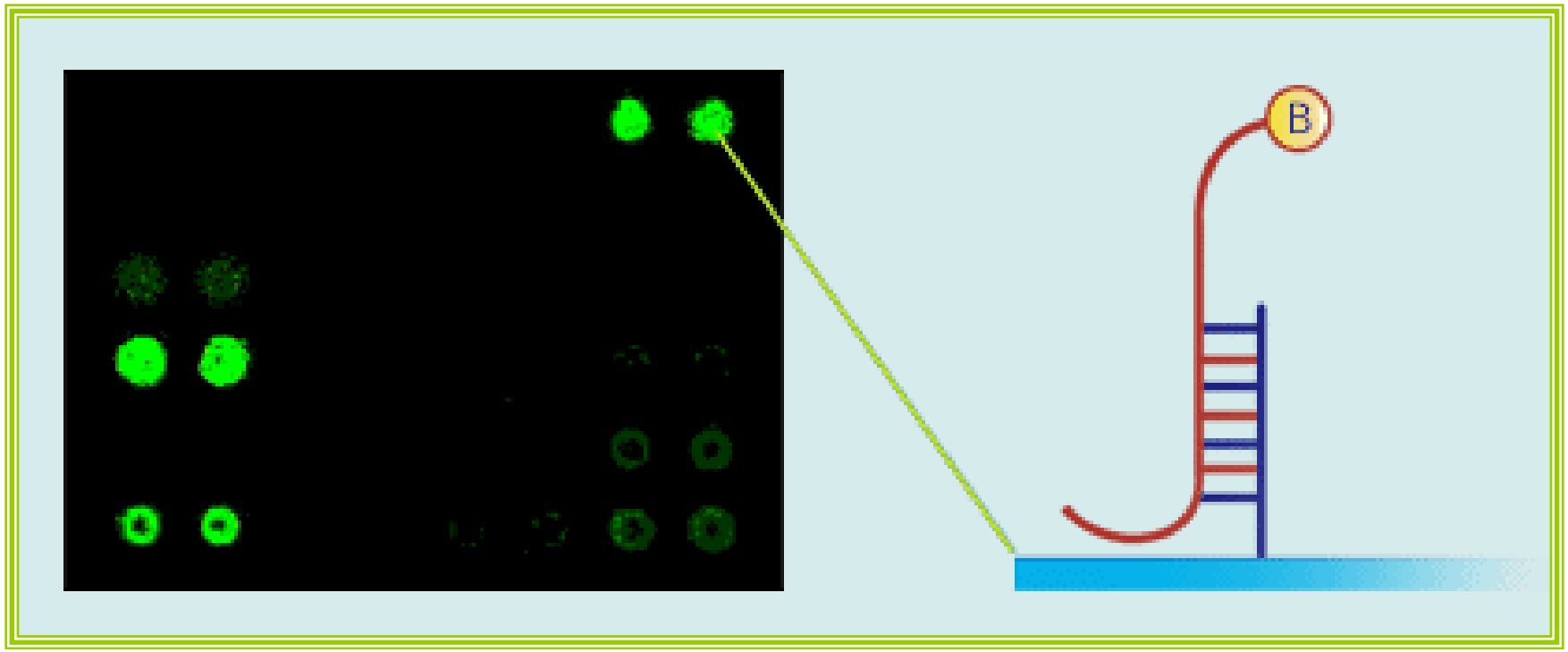
# What is a microarray?

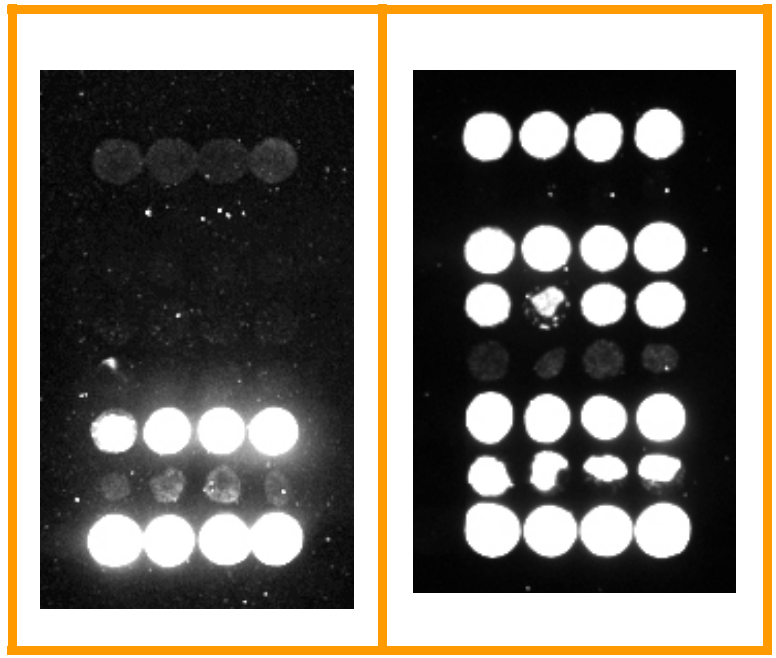



Probes are “printed” on the slide

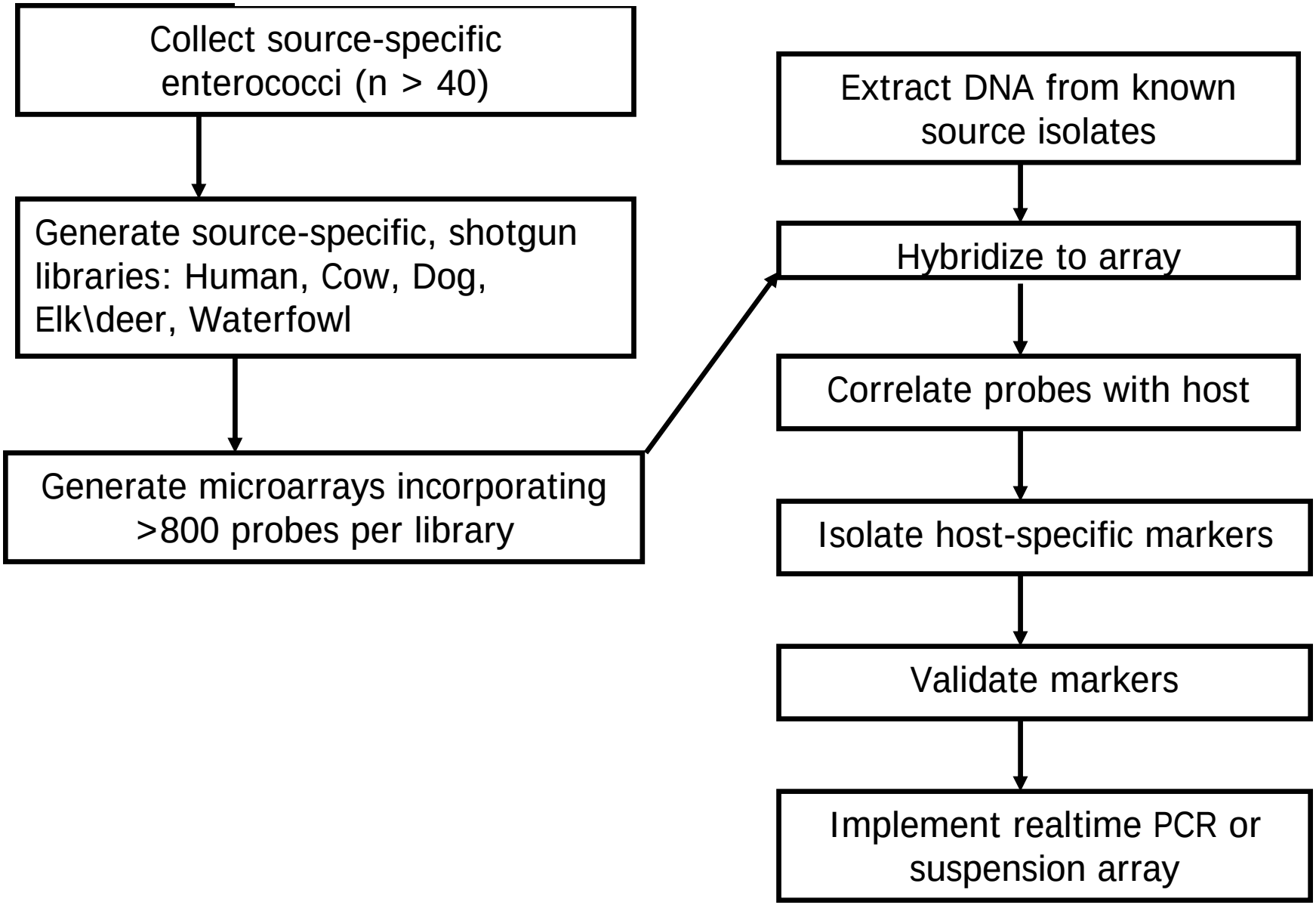
Targets are detected after hybridization


MicroGrid

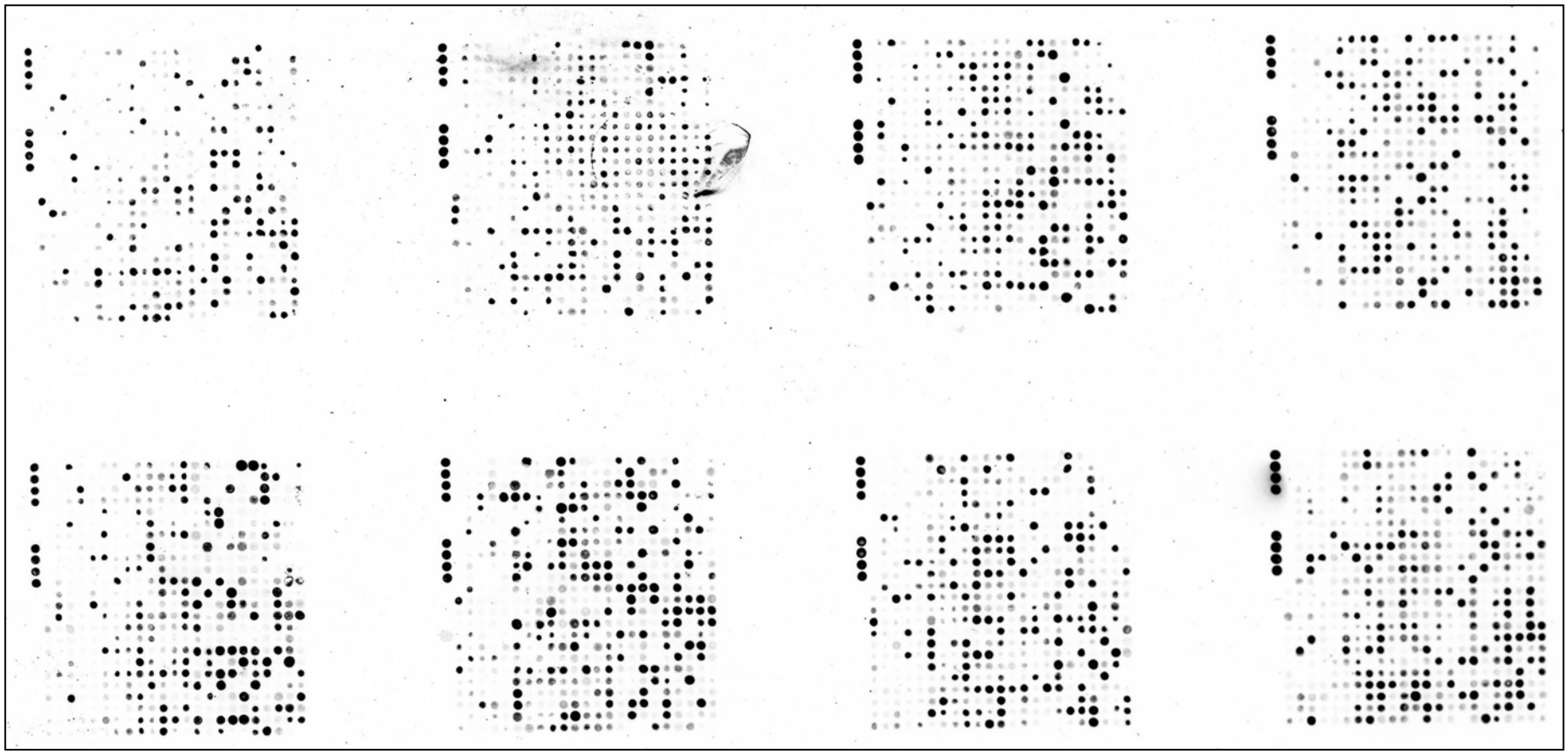

arrayWoRx



# Mixed-Genome Microarrays

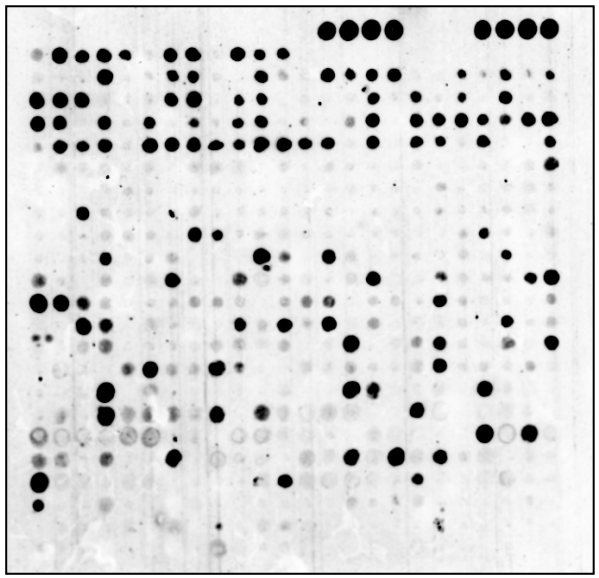


# Example hybridization with mixed-genome, *Enterococcus* microarray

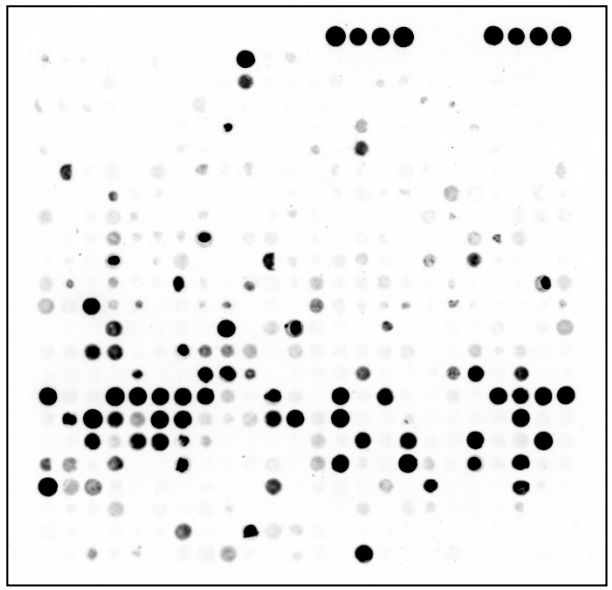


(n = 4,320 probes)

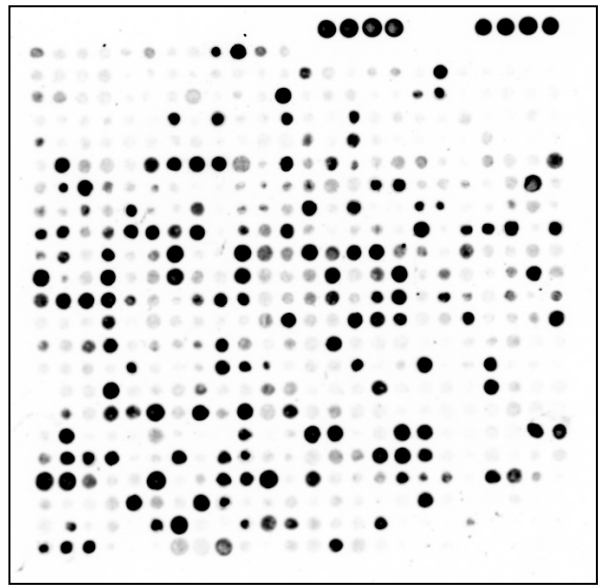
Human isolate



Elk/deer isolate

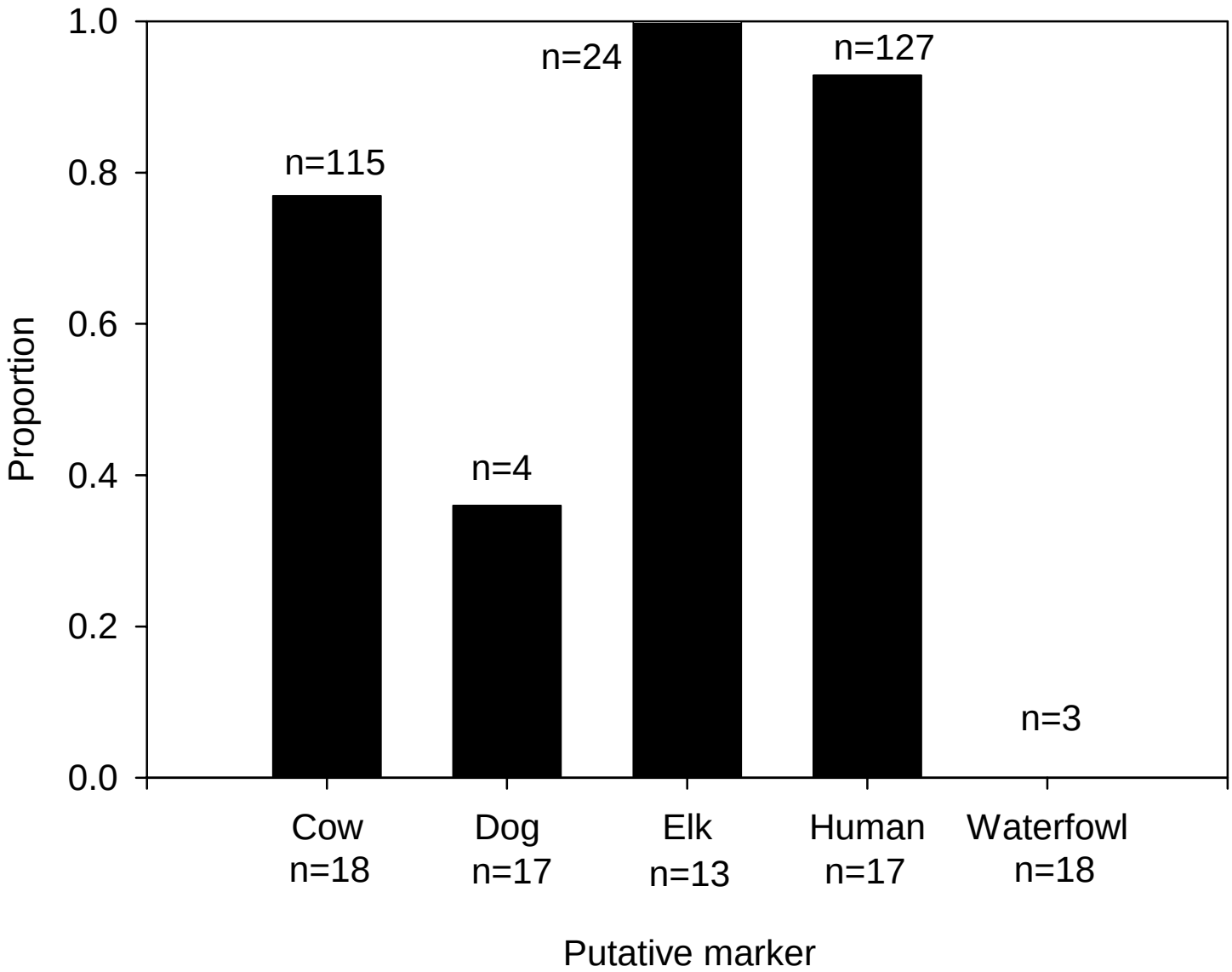


Cow isolate



- Data normalized by subgrid using 16S rDNA probe
- Preliminary data includes 83 hybridizations
- N = 358,560 data points

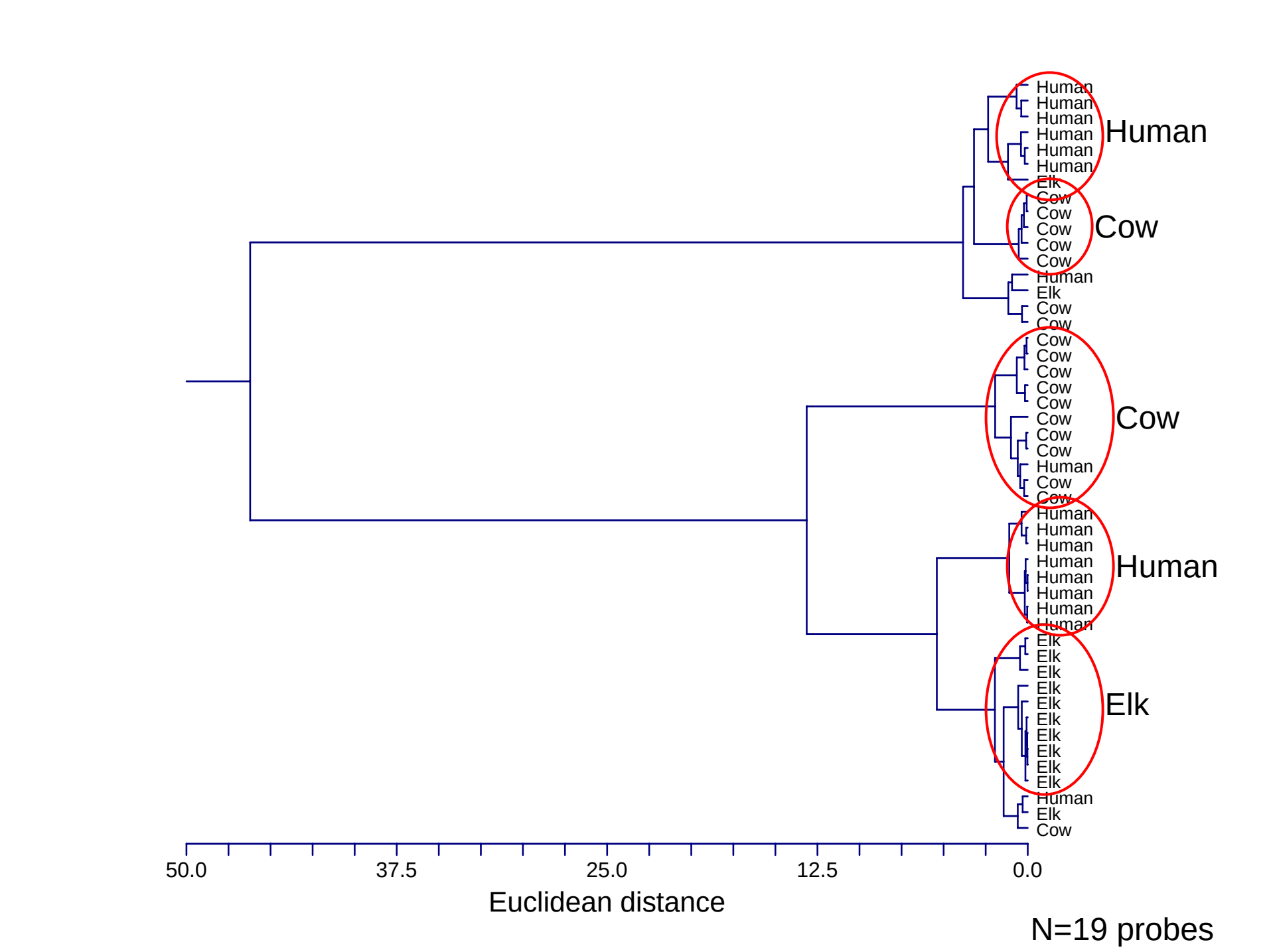
# Proportion of putative markers from host-specific libraries



# Discriminant function analysis

| | Cow | Dog | Elk | Human | Wfowl |
|---|---|---|---|---|---|
| Cow | 14 | 1 | | 2 | 1 |
| Dog | 2 | 12 | | | 3 |
| Elk | | | 13 | | |
| Human | 1 | | | 15 | 1 |
| Wfowl | 3 | 1 | | 1 | 13 |

19 probes; 98.5% correct classification rate
8 probes; 75.9% correct classification rate


Human
Human
Human
Human
Human
Human
Elk
Cow
Cow
Cow
Cow
Cow
Human
Elk
Cow
Cow
Cow
Cow
Cow
Cow
Cow
Cow
Cow
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Human
Human
Elk
Elk
Elk
Elk
Elk
Elk
Elk
Elk
Elk
Elk
Human
Elk
Cow
Human
Cow
Human
Elk
50.0
37.5
25.0
12.5
0.0
Euclidean distance
N=19 probes

# What's next?

- Work plan:
  - Complete additional 200-250 hybridizations
  - Narrow selection (<400 probes)
  - Validate markers against independent isolates
    - Verify host specificity
    - Quantify marker carriage
  - Test with water samples
  - Develop PCR assays (suspension arrays?)

# Summary

- Phenotypic or genotypic markers

- Library-dependent
  - Large effort to prepare
  - Validity over space and time?
  - 50-80% correct classification – good enough?

# Summary

- Library-independent
  - Few markers available
  - Validity still needs to be confirmed
  - Still needs to be cheap and simple

# Acknowledgements

Co-investigators:
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•Tom Besser, VMP & WADDL, Pullman
•Monica Borucki, USDA-ARS, Pullman

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*Needed: fecal samples from around the country.* Please contact: Doug Call, drcall@wsu.edu, 509-335-6313